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Variations in microbial isotopic fractionation during soil organic matter decomposition

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Abstract The soil microbial biomass (SMB) is known to participate in key soil processes such as the decomposition of soil organic matter (SOM). However, its contribution to the isotopic composition of the SOM is not clear yet. Shifts in the ^{13}C and ^{15}N natural abundances of the SMB and SOM fractions (mineralised, water soluble and non-extractable) were investigated by incubating an unamended arable soil for 6 months. Microbial communities were also studied using Fatty Acid Methyl Ester specific isotope analysis. The SMB was significantly ^{13}C and ^{15}N-enriched relative to other fractions throughout the incubation. However, significant isotopic variations with time were also observed due to the rapid consumption of relatively ^{13}C-enriched water soluble compounds. The increase in the difference in SMB and water soluble ^{15}N compositions as the water soluble C/N ratio decreased, indicated a shift from N assimilation to N dissimilation during the incubation. These changes also induced modifications of the microbial community structure. Once the system reached a steady-state (after 1 month), the isotopic trends appeared to corroborate those obtained in long term experiments in the field in that there was a constant microbial isotopic fractionation leading to a ^{13}C and ^{15}N enrichment of the SOM over the long-term. This work also suggests that caution must be exercised when interpreting short term incubation studies since perturbations associated with experimental set-up can have an important effect on C and N dynamics, microbial fractionation of ^{13}C and ^{15}N and microbial community structure.

Keywords ^{13}C · ^{15}N · Isotope fractionation · Soil organic matter · Microbial community

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Introduction

Although most soil organic matter (SOM) is ultimately plant derived, only a small fraction of the yearly litter and root input is incorporated into the stable organic matter pool, most of it after repeated processing by soil microorganisms (Six et al. 2000). By using different NMR spectroscopy techniques, Simpson et al. (2007) and more recently, Miltner et al. (2009) reported that the microbial biomass contribution to SOM formation was far more important than was previously thought. These findings are in line with evidence that microbially derived compounds such as murein, chitin, certain lipids, and

so-called melanins accumulate in soils (Marschner et al. 2008 and references therein). Even carbohydrates and certain peptides produced by soil microorganisms seem to make up a substantial part of the stable subsoil dissolved organic matter (Guggenberger et al. 1994) and can persist in soils for several decades (Gleixner et al. 1999). On the basis of these studies, microorganisms should be considered not only as the drivers or catalysts behind the decomposition process but also as a significant source of stabilized SOM.

Stable isotope analysis (^{13}C and to a lesser degree ^{15}N) is an increasingly used tool for estimating SOM dynamics (Balesdent and Mariotti 1996; Ehleringer et al. 2000). The ^{13}C and ^{15}N natural abundance of SOM is usually higher than that of the plant and fresh litter input (Lichtfouse et al. 1995; Handley et al. 1999; Amundson et al. 2003; Wynn et al. 2005, 2006). Older, more decomposed SOM is ^{15}N- and, less consistently, ^{13}C-enriched compared to less decomposed compounds (Tiessen et al. 1984; Nadelhoffer and Fry 1988; Lichtfouse et al. 1995; Kramer et al. 2003). These results tend to corroborate those obtained in long-term bare-fallow experiments showing significant increases in bulk soil $\delta^{13}C$ and $\delta^{15}N$ in conjunction with decreases in C and N content (Balesdent and Mariotti 1996; Bol et al. 2008). It has been proposed that the isotopic enrichment of the SOM is due to one of two processes: either the preferential degradation of ^{13}C-depleted substrate or an isotopic fractionation during microbial processing (Balesdent and Mariotti 1996). To date, there is no convincing evidence to substantiate the first hypothesis but a recent study has suggested that the microbial biomass itself may be the origin of these isotopic enrichments of the SOM (Dijkstra et al. 2006). The contribution of the ^{13}C or ^{15}N enriched microbial-derived molecules to the isotopic composition of the SOM might be related to the degree and the rate at which these compounds are stabilised, depending on the physical properties of the soil and/or the environmental conditions. Fractionation during microbial processing, however small, may therefore be responsible for significant shifts in the stable isotope composition of the SOM over long time periods (Nadelhoffer and Fry 1994; Kramer et al. 2003). This hypothesis has been used to explain differences in the ^{13}C composition with depth in two soils with similar climate, vegetation and topographic position but different textures (Wynn et al. 2005).

However, neither the underlying mechanisms nor the dynamics of the microbial isotopic fractionation are clearly understood (Ehleringer et al. 2000). It is important to evaluate the magnitude and temporal variations of microbial isotopic fractionation directly in soils because of the widespread use of isotopes in SOM studies. An underlying assumption of SOM dynamics studies using stable isotopes is that the microbial isotopic fractionation is a constant. This assumption has never been verified. The objectives of this study were to (1) quantify the soil microbial ^{13}C and ^{15}N fractionation process, (2) explain the possible link between temporal isotopic variations and C and N dynamics, (3) identify potential relationships between microbial community structure and microbial isotopic fractionation. Thus, we incubated an unamended arable soil for 6 months and measured C and N contents, as well as the natural ^{13}C and ^{15}N abundances of the soil, the microbial biomass, the mineralised, the water soluble and non-extractable fractions of the SOM. We monitored the structure of the microbial communities responsible for these processes by using fatty acid methyl-esters (FAME).

Materials and methods

Soil samples

Samples were obtained from the ploughed layer (0–30 cm) of a cultivated Luvisol from an INRA (Institut National de la Recherche Agronomique) experimental site (Les Closeaux) in Versailles, France. The soil is a silt loam (30% sand, 53% silt, 17% clay), with 13.8 ± 0.6 g kg^{-1} total organic carbon (TOC), 1.35 ± 0.03 g kg^{-1} total nitrogen, and a pH of 6.8. The bulk isotopic compositions were -26.2 ± 0.4‰ and 7.2 ± 0.3‰, for $\delta^{13}C$ and $\delta^{15}N$ (see Eq. 1 below), respectively. The experimental plot had been under C3 plants for at least 50 years and has been under continuous wheat since 1992. Each year, crop residues are returned after harvest and the field is tilled in October. Five soil samples were collected from random locations in the plot in December and sieved moist using a 5 mm mesh sieve. A representative composite sample was formed by bulking all samples. Visible root fragments and plant debris retained on the sieve were discarded. Prior to use, soil samples were pre-incubated at sampling water content

(0.16 g water g^{-1} of oven dry soil corresponding to a matric potential of 100 kPa) for 3 weeks at 20°C.

Incubation

Sub-samples (100 g dry weight equivalent) were amended with 10.6 ml of MilliQ water (Millipore) in order to bring the water content to 0.26 g water g^{-1} oven dry soil (field capacity, a matric potential of 16 kPa). Immediately after adding the water, the sample bottles were flushed with CO_2 free air (19% O_2, 81% N_2). The soil was incubated in serum bottles (565 ml) with Teflon® rubber stoppers crimped on with aluminium seals, at 20°C and in the dark for 6 months. The CO_2 content of the headspace and the $\delta^{13}C$ of the CO_2 were measured after 1, 3, 8, 15, 30, 60, 90 and 180 days incubation. After each CO_2 measurement, soils were destructively sampled for FAME profiling and measuring C, N, ^{13}C and ^{15}N contents of the soil microbial biomass, the mineralised, the water soluble and non-extractable fractions of the SOM. Additional CO_2 measurements were also performed after 45 and 130 days incubation in order to verify that the CO_2 levels in the headspace were such that the system remained aerobic.

Analytical

The CO_2 concentration was determined with a micro-GC (Agilent 3000A, Qplot column). The isotopic composition ($\delta^{13}C$, ‰) of the CO_2–C was determined using a GC (Hewlett-Packard 5890) coupled to an Isotope Ratio Mass Spectrometer (GC-IRMS; Isochrom Optima, Micromass). At each sampling date, samples that were not destructively sampled were flushed with CO_2-free air, sealed and replaced in the incubation chamber. Bottles were weighed before and after the air flush and MilliQ water was added with a syringe through the Teflon® rubber if any loss of water was observed.

The water soluble fraction was determined by shaking subsamples (80 g dry weight equivalent) with 250 ml of MilliQ water overnight (16 h) on a rotary shaker (150 rpm) in the dark. The solutions were then centrifuged (3750 g for 60 min) and the supernatant filtered using a GF/C membrane. The soil was then rinsed twice with 30 ml MilliQ water. A 10 ml aliquot was used to measure the amount of inorganic N contained in the water extract. The remaining extract was freeze-dried for elemental (C, N) and isotopic ($\delta^{13}C$, $\delta^{15}N$) analyses, carried out using an Elemental Analyser coupled to an IRMS (EA-IRMS, NA-1500, Carlo-Erba). The amount of water soluble inorganic N (WSIN) was measured by analysing NO_3^- and NO_2^- with a continuous flow analyser (Skalar San++, Breda, The Netherlands). The amount of NH_4^+ in the extracts was negligible. The water soluble organic N (WSON) was calculated by subtracting the amount of water-soluble inorganic N from the total soluble N measured by elemental analysis. Since no carbonates were found in the soil studied here, the water soluble C was assumed to be mostly in organic form.

The amount of C and N and the isotopic composition of the microbial biomass were measured on the remaining 20 g (dry weight equivalent) subsamples by fumigation-extraction (FE) using 0.03 M K_2SO_4 as an extractant (Gaillard et al. 1999). K_2SO_4 extracts were freeze-dried before EA-IRMS analysis. A C correction factor (K_{EC}) of 0.38 and an N correction factor (K_{EN}) of 0.54 were used (Vance et al. 1987). The ^{13}C and ^{15}N compositions of the microbial biomass were calculated using mass balance (Dijkstra et al. 2006).

FAME profiles were produced following the protocol described in Lerch et al. (2009). Once extracted with methanol and dichloromethane, fatty acids were methylated with BF_3/Methanol and hydroxy-fatty acids were sillilated by using N,O bis(trimethylsilyl)-trifluoroacetamide with 1% of trimethylchlorosilane (Sigma-Aldrich) during 1 h at 70°C. FAME were quantified on a Gas Chromatograph (HP 6890) coupled to a Flame Ionisation Detector (GC-FID) and identified by GC (HP 6890) coupled to an Agilent 5973 Electronic Impact (70 eV) quadrupole Mass Spectrometer (GC-MS). The individual isotopic analysis was carried out using a GC (HP 5890) coupled to an Isochrom III Isotopic Mass Spectrometer (Micromass-GVI Optima) via a combustion interface (GC-c-IRMS). All GC were equipped with the same SGE BPX-5 column (50 m × 0.25 mm × 0.32 μm). Column temperature was programmed at 50°C for 1 min and then ramped at 2°C min^{-1} to 350°C, followed by an isothermal period of 10 min. Fatty acid nomenclature used was that described by Frostegård et al. (1993). Mono-unsaturated and cyclopropyl fatty acids were taken as gram-negative bacteria (G-) biomarkers (O'leary and Wilkinson 1988; Zelles 1999), iso- and anteiso-fatty acids as gram-positive bacteria (G+) biomarkers (O'leary and Wilkinson 1988; Zelles 1999), C18:2(9,12) as a fungal biomarker (Frostegard. 1993;

Zelles et al. 1997) and carboxylic acids with a methyl function on the carbon chain as biomarkers for actinobacteria and in particular *Nocardia* (Zelles et al. 1994).

The remaining fraction of soil after the water and organic solvent extractions was freeze-dried and homogenised. The amount and the isotopic composition of non-extractable organic C (NEOC) and N (NEON) were determined using EA-IRMS as described for the water soluble fraction and the microbial biomass.

Isotopic calculations

Carbon and nitrogen isotope ratios are presented in δ notation, defined as follows:

$$\delta(\%) = \left(\frac{R_{\text{Sample}} - R_{\text{Std}}}{R_{\text{Std}}}\right) \times 1000 \quad (1)$$

where R_{Sample} is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ isotope ratios of the sample and R_{Std} is the $^{13}C/^{12}C$ ratio of the VPDB standard (Coplen 1995) or $^{15}N/^{14}N$ ratio of atmospheric N_2 (Mariotti 1983). Precision of measurements was 0.1 ‰ for δ ^{13}C and 0.2 ‰ for δ ^{15}N.

The derivatisation of the fatty acids introduces one additional carbon which is not present in the parent compound and which alters the original isotope ratio of the fatty acids. The measured isotope ratios of the FAME were corrected for the isotope ratio of the methyl moiety to obtain the isotope ratios of the non-derivatised carboxylic acids as described by Lerch et al. (2007). This was done by using the formula:

$$\delta^{13}C_{\text{FA}} = \frac{(C_n + 1) \times \delta^{13}C_{\text{FAME}} - \delta^{13}C_{\text{MetOH}}}{C_n} \quad (2)$$

where $\delta^{13}C_{\text{FA}}$ is the $\delta^{13}C$ of the fatty acid, C_n is the number of carbons in the fatty acid, $\delta^{13}C_{\text{FAME}}$ is the $\delta^{13}C$ of the fatty acid methyl ester (FAME), and $\delta^{13}C_{\text{MetOH}}$ is the $\delta^{13}C$ of the methanol used for the methylating reaction (−63.2‰).

The overall $\delta^{13}C$ value of FAME ($\delta^{13}C_{\sum\text{FAME}}$) was obtained by weighted average:

$$\delta^{13}C_{\Sigma\text{FAME}} = \left(\sum_{i=1}^{q} \delta^{13}C_i \times C_i\right) / \sum_{i=1}^{q} C_i \quad (3)$$

where C_i is the C amount of each FAME (μg C), $\delta^{13}C_i$ is the isotopic content of each FAME (‰) and q is the total number of FAME.

The difference between the isotopic compositions of two SOM fractions was estimated using the per mil fractionation factor, Δ, which was estimated as the difference in the δ values of two different materials (Fry 2006). Five such ^{13}C per mil fractionation factors were used in this study: the difference between (1) the $\delta^{13}C$ value of the SMB and that of the soil SOC ($\Delta^{13}C_{\text{SMB/SOC}}$), (2) the $\delta^{13}C$ value of the SMB and that of the CO_2 evolved ($\Delta^{13}C_{\text{SMB/Min}}$), (3) the $\delta^{13}C$ value of the CO_2 and that of the SOC ($\Delta^{13}C_{\text{Min/SOC}}$), (4) the $\delta^{13}C$ value of the water-soluble OC and that of the SOC ($\Delta^{13}C_{\text{WSOC/SOC}}$) and (5) the average $\delta^{13}C$ value of the FAME and that of the soil SMB ($\Delta^{13}C_{\sum\text{FAME/SMB}}$). Similarly, three ^{15}N fractionation factors were used: the difference between (1) the $\delta^{15}N$ value of the SMB and that of the bulk soil N ($\Delta^{15}N_{\text{SMB/SN}}$), (2) the $\delta^{15}N$ value of the soil water soluble nitrogen (both organic and inorganic) and that of the of the bulk soil N ($\Delta^{15}N_{\text{WSN/SN}}$), (3) the $\delta^{15}N$ value of the SMB and that of the soil water soluble nitrogen ($\Delta^{15}N_{\text{SMB/WSN}}$).

Mathematical and statistical analyses

Parameters describing changes in CO_2 and the water-soluble fractions (carbon and nitrogen) were obtained by fitting the data with nonlinear regression (SigmaPlot; Systat Software, Inc.). Differences in microbial community structure were analysed by principal components analysis (PCA) using the relative amount of each FAME (expressed in molar percentage) as variables. The isotopic content of the microbial communities was also analysed by PCA, but, in this case, with the $\delta^{13}C$ values of each individual FAME as variables. Significant differences among samples were tested by ANOVA using GenSTAT software (VSN International Ltd.). Time was considered as an independent factor because measurements at different dates were carried out on different samples, due to the destructive sampling regime. All experiments were carried out in triplicate. In addition, there were three analytical replicates for all the isotopic analyses performed with EA-IRMS and GC-IRMS.

Results

C and N dynamics

The amount of non-extractable OC remained stable around 13.3 ± 0.5 mgC g^{-1} soil, a similar value to that of the bulk soil measured before the incubation

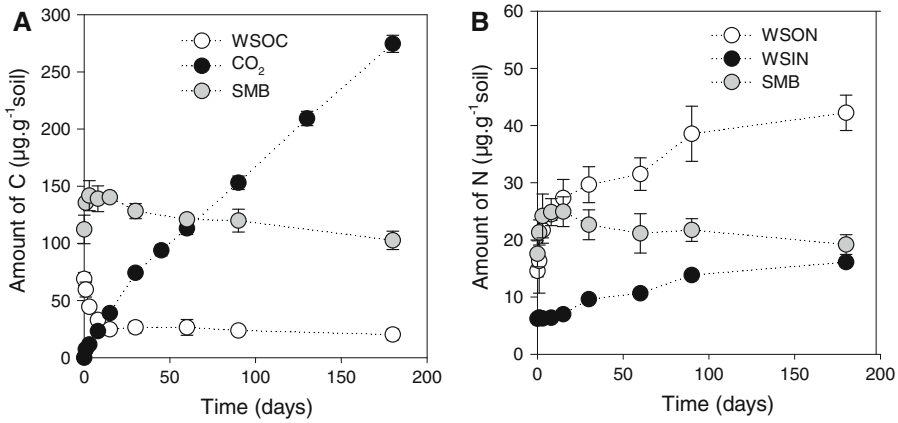


Fig. 1 Evolution of the cumulative CO_2 evolved, Soil Microbial Biomass (SMB), and the water soluble organic carbon (WSOC) expressed in µC g^{-1} soil (**a**) and evolution of the water soluble inorganic (WSIN) or organic (WSON) nitrogen, and the Soil Microbial Biomass (SMB) expressed in µN g^{-1} soil (**b**). Standard deviations correspond to three replicates

(data not shown). The amounts of mineralised, water-soluble and microbial biomass C are presented in Fig. 1a. After 6 months incubation, the total amount of C–CO_2 evolved reached 274 ± 8 µg g^{-1}soil, corresponding approximately to 2% of the soil organic carbon (SOC) of the soil. A significant change in the daily mineralisation rate was observed with time ($P < 0.001$). The cumulative mineralisation curve was fitted ($r^2 = 0.99$, $P < 0.001$) with a first order kinetic equation with 2 compartments: $y = a(1 - e^{-\alpha t}) + b(1 - e^{-\beta t})$, where a and b are the mineralisation potentials and α and β the first order rate constants, respectively for the two compartments of C. The parameters had values of $a = 75$ µgC, $b = 13$ mgC, $\alpha = 0.02$ day^{-1} and $\beta = 8.2 \times 10^{-5}$ day^{-1}, suggesting the presence of a labile and a non-labile compartment. The water soluble organic carbon changed significantly ($P < 0.001$) during the incubation (from 68 ± 6 at the beginning to 20 ± 5 µg.g^{-1} soil at the end of the experiment) and was also fitted with a first order kinetic equation with 2 compartments ($y = a.e^{-\alpha t} + b.e^{-\beta t}$). Here the values of the parameters were a = 42 µgC, b = 27 µgC, $\alpha = 0.28$ day^{-1} and $\beta = 1.5$ 10^{-3} day^{-1}. All the parameter values were significant ($P < 0.05$). The measurement of the microbial C showed a significant change with time ($P < 0.05$). The initial amount of microbial C was 118 ± 2 µg.g^{-1} soil. It increased during the first 3 days of the incubation to reach a maximum of 141 ± 3 µg g^{-1} soil. After 15 days incubation, the amount of microbial C decreased monotonically to a minimum of 106 ± 12 µg.g^{-1} soil reached at the end of the incubation.

The amount of non-extractable N remained stable around 1.1 ± 0.1 mgN g^{-1} soil, a similar value to that of the bulk soil measured before the incubation (data not shown). The amounts of water-soluble inorganic and organic N and microbial biomass N are presented in Fig. 1b. Over the period of the incubation, 1.2% of the soil organic N was mineralised to NO_3^-, or NO_2^- and 2.1% rendered water soluble in organic form. As a result, the C/N ratio of the water soluble fraction varied from 3.3 ± 0.3 at day 0 to 0.5 ± 0.1 at day 15 and remained at this value for the remainder of the incubation. The C/N ratio of the non-extractable and the microbial biomass fractions did not change significantly with time (12.3 ± 1.2 and 6.1 ± 0.6, respectively). The water-soluble inorganic N (WSIN) curve was fitted ($r^2 = 0.95$, $P < 0.001$) with a first order kinetic equation with 1 compartment: $y = a(1 - e^{-\alpha t}) + y_0$, where a is the mineralisation potential, α the first order rate constant and y_0, the initial amount of water-soluble inorganic N. The parameter values were a = 1.3 mgN, $\alpha = 5.9 \times 10^{-5}$ day^{-1} and $y_0 =$ 6.6 µgN. The water-soluble organic N (WSON) curve was fitted ($r^2 = 0.99$, $P < 0.001$) with a first order kinetic equation with 2 compartments: $y = a(1 - e^{-}\alpha^{t}) + b(1 - e^{-\beta t}) + y_0$. The parameter values were a = 9.8 µgN, b = 23.4 µgN, $\alpha = 0.33$ day^{-1}, $\beta = 8.7$ 10^{-3} day^{-1} and $y_0 = 14.2$ µgN. The measurement of the microbial N showed a significant change with time ($P < 0.05$). The initial amount of microbial N was 17.6 ± 2.2 µg g^{-1} soil. Maximum values were reached between 3 and 15 days (24.2 ± 3.8 to 24.9 ± 2.5 µg g^{-1} soil). After 15 days

of incubation, the amount of microbial C decreased monotonically to 19.2 ± 1.7 μg g^{-1} soil reached at the end of the incubation.

Changes in $\delta^{13}C$ and $\delta^{15}N$ values

The ^{13}C contents of the CO_2 evolved, the microbial biomass, the water-soluble OC and the non-extractable OC throughout the experiment are given in Fig. 2a and the fractionation factors in Table 1. The $\delta^{13}C$ value of the non-extractable OC remained constant (average value of −26.2 ± 0.3‰) during the 6 month incubation and did not differ significantly from that of the bulk soil measured before the incubation (−26.2 ± 0.4‰). Analysis of variance showed a significant change with time in the ^{13}C content of the headspace CO_2 ($P < 0.001$), the microbial biomass ($P < 0.005$) and the water-soluble OC ($P < 0.005$). The microbial biomass was always the most ^{13}C-enriched fraction (Table 1). The CO_2 evolved was significantly ^{13}C-enriched compared to SOC up to the 3rd day. Thereafter, it declined to become the most ^{13}C-depleted fraction after 30 days. During the first 3 days of the incubation, the ^{13}C enrichment of the water-soluble OC was significantly higher than that of the bulk soil organic C, as shown by the $\Delta_{WSOC/SOC}$ in Table 1. Subsequently, there were no significant differences between the two fractions. The isotopic composition of the microbial biomass and the CO_2 varied similarly: a maximum was reached after 3 days incubation (−23.6 ± 0.3‰ and −24.6 ± 0.2‰, respectively) and thereafter slowly decreased to a minimum (−25.5 ± 0.3‰ and −27.7 ± 0.1‰, respectively) after 180 days of incubation. However, there were significant differences in the $\Delta_{SMB/Min}$, principally between the $\Delta_{SMB/Min}$ of day 3 and those of the days that followed. There were also significant differences between the $\Delta_{SMB/Min}$ of day 1 and those of days 8 and 15 (Table 1).

The $\delta^{15}N$ values of the water soluble N (organic and inorganic), of the microbial N, and the non-extractable N throughout the experiment are presented in Fig. 2b. The $\delta^{15}N$ value of the non-extractable N (7.5 ± 0.1‰) was not significantly different from that of the bulk soil measured before the incubation (7.2 ± 0.3‰). The microbial biomass was significantly ($P < 0.005$) ^{15}N-enriched relative to the bulk soil from day 15 onwards, whereas the water soluble N was significantly ($P < 0.001$) ^{15}N-depleted from day 3 onwards (Table 2). Significant changes with time in the ^{15}N content of the microbial biomass ($P < 0.005$) and the water soluble N were detected by ANOVA. The $\delta^{15}N$ value of the microbial N increased significantly between days 15 and 30 and remained stable thereafter, whereas the $\delta^{15}N$ value of the water soluble N decreased significantly between days 1 and 3 and again between days 7 and 15 (Fig. 2b). Table 2 shows that the $\Delta^{15}N_{SMB/SN}$ increased significantly with time after day 15 while $\Delta^{15}N_{WSN/SN}$ decreased significantly after day 3. As a consequence, $\Delta^{15}N_{SMB/WSN}$ increased significantly with time. It has been suggested that the relationship between the the ^{15}N enrichment of the microbial biomass relative to that of the water soluble fraction and C/N ratio of the water soluble fraction is an indicator of change in microbial N metabolism (Dijkstra et al. 2008). Here, a significant linear regression ($r^2 = 0.66$, $P < 0.001$, $y = 3.35 - 1.17x$) was also found between C/N ratio of the water soluble fraction and $\Delta^{15}N_{SMB/WSN}$. However, the relationship between these two variables was

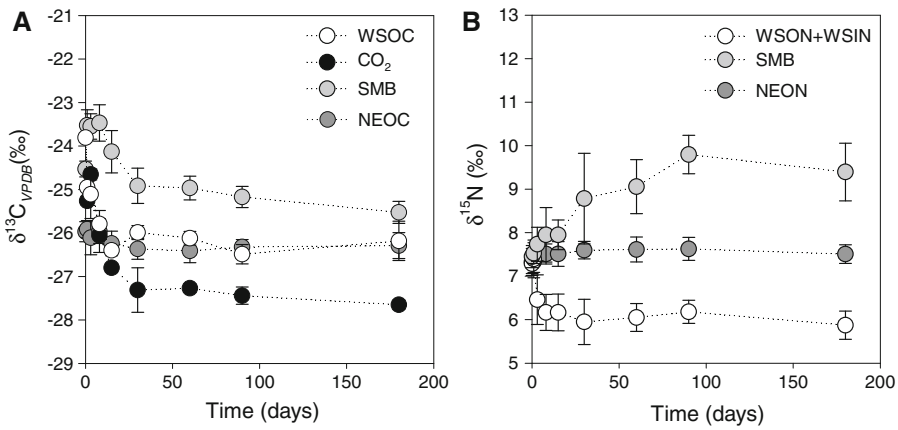


Fig. 2 $\delta^{13}C$ values of the instantaneous CO_2 evolved, the Soil Microbial Biomass (SMB), water soluble (WSOC) and non-extractable (NEOC) organic C (**a**) and $\delta^{15}N$ values of the SMB, the Water Soluble Organic and Inorganic (WSON + WSIN), and the non-extractable organic N (**b**). Standard deviations correspond to three replicates

Table 1 Change of ^{13}C per mil fractionation factors (‰) between the SMB and the CO_2 evolved ($\Delta^{13}C_{SMB/Min}$), the SMB and the SOC ($\Delta^{13}C_{SMB/SOC}$), the CO_2 and the SOC ($\Delta^{13}C_{Min/SOC}$), the WSOC and the SOC ($\Delta^{13}C_{WSOC/SOC}$) and FAME and the SMB ($\Delta^{13}C_{\sum FAME/SMB}$) with time

Time	$\Delta^{13}C_{SMB/Min}$	$\Delta^{13}C_{SMB/SOC}$	$\Delta^{13}C_{Min/SOC}$	$\Delta^{13}C_{WSOC/SOC}$	$\Delta^{13}C_{\sum FAME/SMB}$
0	–	1.7 ± 0.2	–	2.4 ± 0.1	−1.6 ± 0.1
1	1.7 ± 0.4	2.7 ± 0.4	0.9 ± 0.1	1.3 ± 0.0	−2.1 ± 0.3
3	1.1 ± 0.3	2.6 ± 0.3	1.5 ± 0.1	1.1 ± 0.1	−2.0 ± 0.3
8	2.6 ± 0.6	2.7 ± 0.4	0.1 ± 0.3	0.4 ± 0.3	−2.0 ± 0.3
15	2.7 ± 0.4	2.1 ± 0.5	−0.6 ± 0.1	−0.2 ± 0.0	−1.7 ± 0.7
30	2.4 ± 0.4	1.3 ± 0.4	−1.1 ± 0.1	0.2 ± 0.4	−1.4 ± 0.5
60	2.3 ± 0.1	1.2 ± 0.0	−1.1 ± 0.1	0.1 ± 0.5	−1.9 ± 0.2
90	2.3 ± 0.4	1.0 ± 0.2	−1.2 ± 0.2	−0.3 ± 0.2	−1.8 ± 0.3
180	2.1 ± 0.2	0.7 ± 0.3	−1.5 ± 0.1	0.0 ± 0.2	−1.7 ± 0.3
LSD	0.7	0.6	0.3	0.4	0.6

Standard deviations correspond to 3 replicates. Least Significant Difference (LSD) was calculated by the ANOVA

Table 2 Change of ^{15}N per mil fractionation factors (‰) between the SMB and the soil bulk N ($\Delta^{13}C_{SMB/SN}$), the SMB and the WSN ($\Delta^{13}C_{SMB/WSN}$), the WSN and the soil bulk N ($\Delta^{13}C_{WSN/SN}$) with time

Time	$\Delta^{15}N_{SMB/SN}$	$\Delta^{15}N_{SMB/WSN}$	$\Delta^{15}N_{WSN/SN}$
0	0.3 ± 0.3	0.2 ± 0.2	0.1 ± 0.1
1	0.3 ± 0.3	0.1 ± 0.6	0.2 ± 0.4
3	0.5 ± 0.3	1.3 ± 0.8	−0.7 ± 0.6
8	0.8 ± 0.6	1.8 ± 0.2	−1.0 ± 0.4
15	0.8 ± 0.4	1.8 ± 0.3	−1.1 ± 0.3
30	1.6 ± 1.1	2.8 ± 1.1	−1.3 ± 0.2
60	1.9 ± 0.6	3.1 ± 0.8	−1.2 ± 0.2
90	2.6 ± 0.4	3.6 ± 0.5	−1.1 ± 0.2
180	2.2 ± 0.3	3.5 ± 0.2	−1.4 ± 0.2
LSD	0.9	1.1	0.5

Standard deviations correspond to 3 replicates. Least Significant Difference (LSD) was calculated by the ANOVA

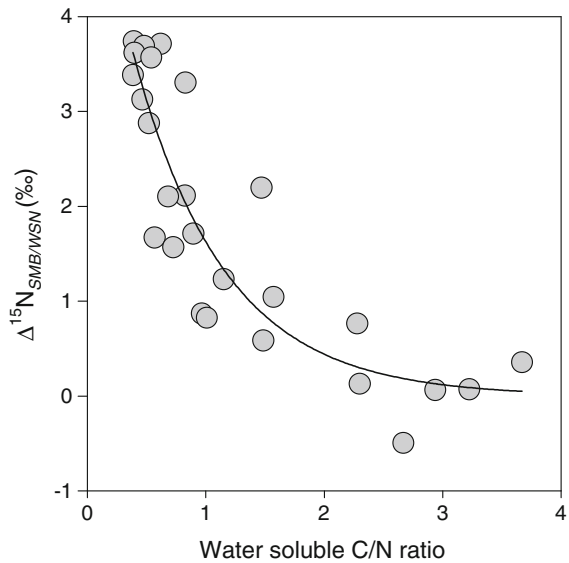


Fig. 3 Non-linear relationship between the microbial biomass ^{15}N enrichment relative to the water soluble fraction ($\Delta^{15}N_{SMB/WSN}$, ‰) and C/N ratio of the water soluble fraction. Symbols are individual measurements at every date of sampling

better described ($r^2 = 0.81$, $P < 0.001$) by using an exponential equation: $y = a(1 - e^{-\alpha t})$ with $a = 5.98$ and $\alpha = 1.30$ (Fig. 3).

FAME profiles

FAME profiles contained 34 different fatty acids. Although the proportion of each FAME varied with time ($P < 0.001$), a general pattern was preserved throughout the incubation: C16:0 was always the most dominant FAME identified with a contribution of at least 20%, C18:2(9,12) and the C18:1(9)cis were the two other major FAME with a contribution of 15% each; the total contribution of C12:0, C14:0, i-C15:0, a-C15:0, C15:0, i-C16:0, C16:1(9)cis, C16:1(11), BrC17:0, 10Me-C16:0, i-C17:0, Cyc C17:0, C17:0, C18:1(9) trans, C18:0, Cyc C19:0, C19:0, C20:0 accounted for about 45% of the total FAME, and the total contribution of C11:0, C13:0, a-C17:0, C17:1(9), 10Me–C17:0, 10Me–C18:0 and all hydroxy-FAME was less than 5% of the total

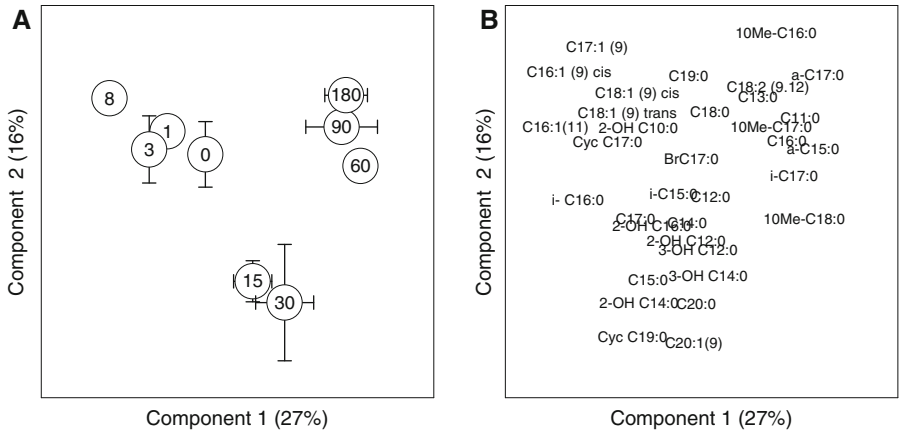


Fig. 4 Scores (**a**) and loadings (**b**) of the 2 main components of the PCA, representing 43% of the variability. Variables are relative abundance of 34 FAME detected between day 0 to day 180 of the incubation. Standard deviations of correspond to three replicates of sampling dates

FAME. The first two ordination axes (representing 43% of the total variability in the FAME profiles) of the principal components analysis are presented in Fig. 4. Along the first ordination axis, principal component scores clustered into three groups related to sampling date, indicating a change in the microbial population structure with time. The 1st cluster included samples from days 0 to 8 and was characterised by a high contribution of monoenoic unsaturated FAME characteristic of Gram-negative bacteria. The 2nd cluster (days 15 and 30) was characterised by the contribution of hydroxy-FAME. The 3rd cluster (2, 3 and 6 months) was characterised by the predominance of branched saturated FAME characteristic of Gram-positive bacteria and the polyunsaturated FAME C18:2(9,12) characteristic of fungi. The second ordination axis separated FAME profiles obtained on days 15 and 30 from the rest of the sampling days. There was a significant linear relationship between the first PC component and $\Delta^{13}C_{SMB/SOC}$ ($r^2 = 0.57$, $P < 0.001$, $y = 5.13 - 2.88x$) and between the first PC component $\Delta^{15}N_{SMB/WSN}$ ($r^2 = 0.73$, $P < 0.001$, $y = 1.90 + 0.39x$) (Fig. 7a and c, respectively).

δ^{13}C values of FAME

The isotopic compositions of 25 FAME were measured. The minor FAME such as C11:0, C13:0, a-C17:0, C17:1(9), 10Me–C17:0, 10Me–C18:0 and all hydroxy-FAME were present in insufficient quantity for a reliable measurement of their isotopic signature. The overall δ^{13}C value of FAME ($\delta^{13}C_{\sum FAME}$) during the incubation was -26.1 ± 0.7‰. A number of FAME were significantly depleted in ^{13}C relative to the overall average value (C14:0, C15:0, C16:1(11), C16:0, C18:0, C18:1(9)cis, C18:1(9)trans,cycC19:0, C19:0 and C20:0) whereas others (C12:0, iC15:0, aC15:0, iC16:0, iC17:0, aC17:0, cycC17:0, C17:0) were significantly enriched. The overall δ^{13}C value of FAME ($\delta^{13}C_{\sum FAME}$) changed significantly with time ($P < 0.001$): it increased to a maximum of -25.1‰ on day 3 and subsequently decreased to a minimum of -27.2‰ at the end of the incubation (Fig. 5). From

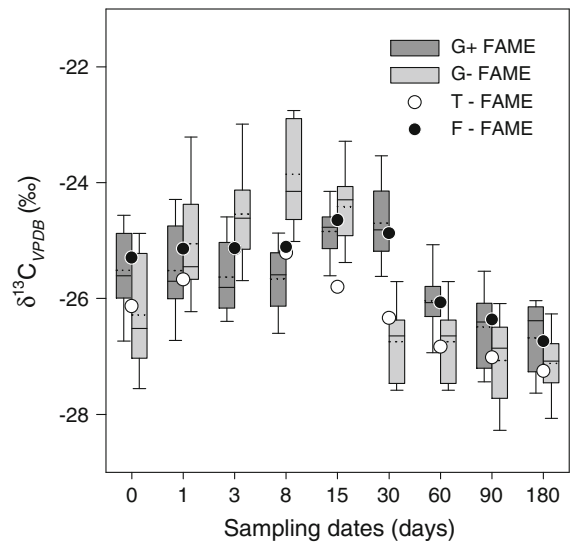


Fig. 5 Change of δ^{13}C values of FAME associated to Gram-positive bacteria (G+FAME), Gram-negative bacteria (G-FAME), fungi (F-FAME) and the overall microbial populations (T-FAME). At each date, the box plots were constructed with 9 and 7 FAME ^{13}C signatures representing G+FAME and G-FAME bacteria, respectively. Dash lines represent the mean of each group. The fungal δ^{13}C signatures (F-FAME) were obtained from $\delta^{13}C_{C18:2(9,12)}$, while δ^{13}C signatures of the overall microbial population (T-FAME) was calculated using all FAME ($\delta^{13}C_{\sum FAME}$). The isotopic signatures of each FAME were the mean of three incubation replicates

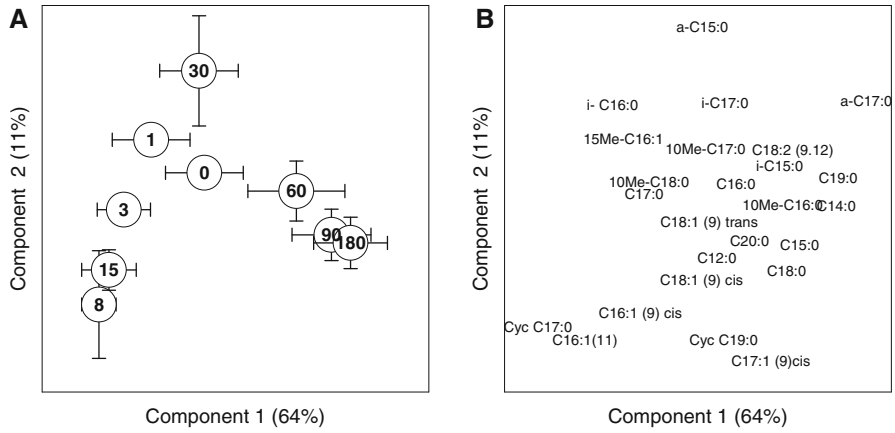


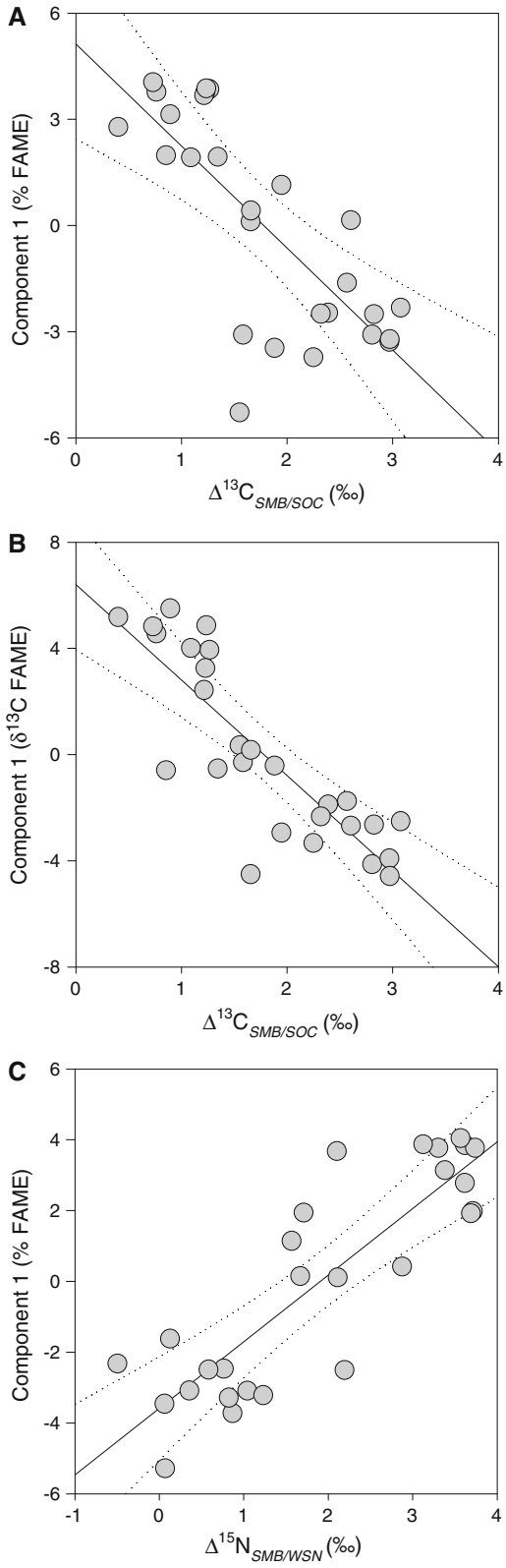
Fig. 6 Scores (**a**) and loadings (**b**) of the 2 main components of the PCA, representing 85% of the variability. Variables are the $\delta^{13}C$ values of FAME ($n = 24$) from day 0 to day 180 of incubation. Standard deviations of correspond to three replicates of sampling dates

30 days onwards, the overall $\delta^{13}C$ signature of gram-positive bacteria FAME was higher than that of gram-negative bacteria FAME. The $\delta^{13}C$ signature of the fungal FAME C18:2(9,12) was significantly ($P < 0.001$) enriched compared to the overall FAME and followed the same trend as that of the gram-positive FAME throughout the incubation period. The difference between the average $\delta^{13}C$ value of the FAME and that of the SMB ($\Delta^{13}C_{\sum FAME/SMB}$) during the 6 months was estimated at $-1.8 \pm 0.5‰$ (Table 1). The first 2 components (75% of variability) of the PCA performed with the $\delta^{13}C$ values of each FAME as variables are presented in Fig. 6. The principal component scores showed significant differences among sampling dates, indicating a change in the isotopic signature of FAME with time. Inspection of the principal component loadings of the first ordination axis indicated that it was related to the overall evolution of the isotopic composition of all FAME (Fig. 5). Between days 1 and 15, the ^{13}C contents of all the FAME generally increased and decreased thereafter. The 2nd component separated samples according to the maximum $\delta^{13}C$ values of the FAME. The isotopic composition of fatty acids characteristic of Gram-negative bacteria were maximal on days 3, 8 and 15 while the fatty acids that were most ^{13}C enriched on day 30 were characteristic of Gram-positive bacteria. There was a significant relationship between the first principal component and $\Delta^{13}C_{SMB/SOC}$ ($r^2 = 0.71$, $P < 0.001$, $y = 6.40 - 3.60x$) (Fig. 7b).

Discussion

Relationship between microbial ^{13}C-fractionation and C dynamics

The consistent ^{13}C-enrichment (0.7–2.7‰, Table 1) of the soil microbial biomass (SMB) relative to the bulk soil (SOC) was at the lower end of the range of values (1.6–5.6‰) observed in other studies (i.e. Ryan and Aravena 1994; Qian et al. 1997; Gregorich et al. 2000; Šantrůčková et al. 2000; Dijkstra et al. 2006). A number of studies carried out with fungal (Gleixner et al. 1993; Hobbie et al. 1999; Högberg et al. 1999) or bacterial pure cultures (Coffin et al. 1989; Hall et al. 1999; Hunkeler et al. 2001; Lerch et al. 2007) have shown a ^{13}C enrichment of the microbial biomass relative to the substrates consumed. This phenomenon has been related to a kinetic isotope effect during the decarboxylation of pyruvate, resulting in a ^{13}C depletion of the CO_2 compared to the substrate used (see review of Ghashghaie et al. 2003 and references therein) and leading to an enrichment of the respiring organism, in this case the microbial biomass. However, the biomass is not always ^{13}C-enriched relative to the substrate despite the respiration CO_2 being ^{13}C-depleted (Blair et al. 1985; Wick et al. 2003), suggesting that this fractionation may not be universal (but rather microorganism or substrate-dependant). For example, ^{13}C-depleted respiration CO_2 can also be explained by fractionation during the



◀ **Fig. 7** Relationships between the microbial biomass-soil organic carbon ^{13}C fractionation factor ($\Delta^{13}C_{SMB/SOC}$, ‰) and the first principal component scores of FAME profiles (**a**), relationship between $\Delta^{13}C_{SMB/SOC}$ and the first principal component scores of FAME $\delta^{13}C$ signatures (**b**) and relationship between the microbial biomass ^{15}N enrichment relative to the water soluble fraction ($\Delta^{15}N_{SMB/WSN}$, ‰) and the first principal component scores of FAME profiles (**c**). The solid line represents the regressions and the dotted lines the 99% confidence intervals. Symbols are individual measurements at every date of sampling

conversion of acetyl phosphate to acetyl-CoA leading to ^{13}C-depleted material entering the Krebs cycle and ^{13}C-enriched acetate being secreted, therefore without enrichment of the biomass (Blair et al. 1985).

From day 8 of the incubation onwards, there was a significant decrease in the ^{13}C-enrichment of the SMB relative to SOC but no significant change was observed in the ^{13}C-enrichment of the SMB relative to CO_2. This combination of events might be explained by changes in the metabolic pathways used to consume the substrate available, with concomitant changes in isotopic fractionation. However, the stability of the ^{13}C-enrichment of the SMB relative to CO_2 during this period suggests that the rate-limiting metabolic steps remained relatively constant.

Another explanation is that the most labile C, consumed by the microbial communities at the beginning of the incubation, was ^{13}C-enriched relative to the substrate consumed subsequently and also relative to the overall SOC (non-extractable C or bulk soil). Were this the case, then the ^{13}C signature of the SMB would be expected to be initially higher than that of the SOC and decline as the substrate consumed became more ^{13}C-depleted. This is what we observed in our experiment. This scenario was corroborated by the similar changes over time in the isotopic composition of the SMB, CO_2 and the water-soluble OC (all more enriched than the NEOC or the bulk soil) at the beginning of the experiment, suggesting that the WSOC was initially the most consumed C fraction. Furthermore, during the period of most intensive microbial activity (days 1–15), there was a rapid decline in both the amount and ^{13}C abundance of the WSOC. The changes in ^{13}C abundance of the WSOC were mirrored by changes in the ^{13}C abundance of the SMB ($\Delta^{13}C_{SMB/SOC}$ did not change significantly with time), even though no

significant relationship was found between these 2 variables.

After the period of intensive microbial activity, the isotopic composition of the WSOC tended towards that of the NEOC, suggesting that microorganisms used another fraction of SOM, i.e. NEOC. This is confirmed by the kinetic analysis of the C dynamics which indicated that respiration was best described by a model with two organic C pools, one with a rapid turnover and one with a slower turnover.

Although the water-soluble OC contained some of the labile C, there was not a direct correspondence between the two. The non-linear equation fitted to the water-soluble OC suggested that two compartments (0.3% and 0.2% of the SOC, respectively) were distinguishable, one relatively labile and the other relatively stable, the turnovers of which were 4 days and 1.8 years, respectively. These data are consistent with those obtained on agricultural soils by Kalbitz et al. (2003a). The water-soluble OC is likely made of different molecular families that are more or less degradable, such as carbohydrates and aromatic molecules, respectively (Kalbitz et al. 2003b). Carbohydrates are known to be ^{13}C-enriched relative to other cell compounds (Glaser 2005; Derrien et al. 2006). Therefore, a preferential uptake of carbohydrates from the water-soluble fraction might explain a part of the early ^{13}C-enrichment of the microbial biomass. These molecules could have been rendered bioavailable by the sample treatment prior to and at the beginning of the experiment. Our isotopic results strongly suggest a change in the nature of substrate consumed by the microbial communities during the incubation leading to variations in the isotopic composition of the SMB. Were this to be confirmed, then the ^{13}C enrichment of the SMB relative to that of the SOC might prove to be an interesting indicator of the type of substrate consumed by soil microbial communities.

Relationship between ^{15}N microbial fractionation and N dynamics

From day 3 onwards, the δ^{15}N value of the soil microbial biomass (SMB) was greater than that of the water soluble and the non-extractable fractions. Heterotrophic organisms are often ^{15}N-enriched in comparison to their substrates. Significant ^{15}N-enrichment of the biomass along the food chain has long been documented (e.g. De Niro and Epstein 1981; Minagawa and Wada 1984). However, this phenomenon has only recently been demonstrated for soil micro-organisms (Dijkstra et al. 2006; Pörtl et al. 2007). The $\Delta^{15}N_{SMB/SN}$ values obtained here stabilised (from day 30 onwards) at a value within the range of +1.4‰ to +5.9‰ observed in various soils by these authors. Prior to that, during the period of intense microbial activity, the $\Delta^{15}N_{SMB/SN}$ values were somewhat lower. As the ^{15}N composition of an organism is a result of the δ^{15}N value of its substrates but also of any fractionation that might occur during N processing (Robinson 2001), the significant increase in the microbial ^{15}N abundance relative to the other soil fractions is likely to have been caused either by a change in the source of δ^{15}N value of the N utilized by soil microorganisms and/or a change in N metabolism.

There is little evidence for the first hypothesis as the δ^{15}N values of the other N fractions remained stable or decreased slightly during the incubation (Fig. 2b). Caution must be exercised however, when interpreting the water soluble fraction because it may contain many different forms of N, each with different δ^{15}N values (e.g.; Houlton et al. 2007). With regard to the soluble organic N fraction, it has been suggested that this is of microbial origin (Pörtl et al. 2007). The fact that the WSON changed most rapidly during the period of intensive microbial activity (Fig. 1a) tends to corroborate this. Therefore, the WSON δ^{15}N value is unlikely to be higher than that of the biomass or to increase with time (Pörtl et al. 2007), meaning that the δ^{15}N-enrichment of the biomass cannot come from the use of this fraction.

The use of the inorganic N is unlikely to have led to enrichment either: as reported by Högberg (1997), the fractionation of N isotopes during the first step of SOM mineralisation is small relative to that occurring during ammonia volatilization, nitrification or denitrification. A consequence of this is that any gaseous loss of N from the system would lead to an increase of the δ^{15}N value of the remaining inorganic N in solution available to microorganisms (Mariotti et al. 1981; Handley and Raven 1992). The pH of the soil studied (6.8) and the aerobic conditions in which the incubation was conducted both suggest that gaseous loss (ammonia volatilisation and denitrification) was likely to be negligible. Although this is confirmed by

the substantial production of nitrites and/or nitrates (which reached approximately 1% of the total soil N during the 6 month incubation; Fig. 1b), gaseous loss cannot be totally ruled out. Since soil microorganisms preferentially immobilize ammonium compared to nitrates in arable soils (Rice and Tiedje 1989; Recous et al. 1990) and there is little isotopic fractionation during the mineralisation of organic N to ammonium (Högberg 1997), one would therefore expect the $\delta^{15}N$ value of the mineral N used by the microbial communities to broadly reflect that of the soil organic N. The preferential uptake of ammonia would also explain the $\delta^{15}N$ of the water soluble extract, containing relatively more ^{15}N-depleted nitrites and/or nitrates.

The second hypothesis explaining the increase of ^{15}N content of the SMB may involve a change in N metabolism. Both N assimilation and dissimilation are concurrent in soils. Net assimilation of N occurs when organic matter undergoing microbial decomposition has an N content that is insufficient to meet the N demand of the microorganisms. On the other hand, when C availability is low, organic molecules containing N are used as a source of C and energy by micro-organisms and the excess N is released from the cells in mineral form (Dijkstra et al. 2008). Fractionation occurring during the processes of dissimilation and export results in a preferential loss of the light isotope. Therefore, if the magnitude of nitrogen dissimilation in microbial communities changes, then the ^{15}N-enrichment of the microbial biomass is also likely to change. Dijkstra et al. (2008) suggested that the linear relationship between the C/N ratio of the water soluble fraction and the $\Delta^{15}N_{SMB/WSN}$ indicates a shift from N assimilation to N dissimilation.

Despite the fact that the enrichment was lower than that reported by Dijkstra et al. (2008), a negative relationship was also found here; although the relationship appeared to be non-linear in this case. The difference between the relationship obtained here and that found by Dijkstra et al. (2008) may be due to the dynamic nature of this study. The relationship was measured in the same soil over a period of 6 months here, whilst Dijkstra et al. (2008) measured the relationship at a single time point but in many locations, and therefore at a larger spatial scale. The non-linear nature of the relationship obtained in this study can be explained by changes in microbial community structure, leading to different assimilation/dissimilation ratios. This hypothesis is corroborated by the significant relationship between $\Delta^{15}N_{SMB/WSN}$ and the first principal component axis of the FAME data (Fig. 3b). Such a change in N metabolism would explain the sharp increase in the $\delta^{15}N$ value observed between days 15 and 30 (Fig. 2b; Table 2). Prior to day 15, C availability was higher, as indicated by the water-soluble OC (Fig. 1a) as was the microbial N requirement for growth and maintenance. During this period, mineral N might be more assimilated by micro-organisms and this process may compensate for fractionation during N dissimilation. Isotopic analyses of the water soluble inorganic N (nitrates and ammonium) would be necessary to confirm this phenomenon.

Relationship between microbial ^{13}C-fractionation and community structure

The FAME profiles suggest that at the beginning of the incubation, when the labile substrate was most abundant and the respiration rate was the highest, the microbial communities were dominated by Gram-negative, copiotrophic bacteria. Gram-negative bacteria are known to proliferate after the addition of organic material (Bossio and Scow 1998; Kramer and Gleixner 2006; Elfstrand et al. 2008). It is conceivable that the labile organic matter available at the beginning of the incubation was similar to added fresh organic matter with similar effects on microbial community structure. There was a transition in the microbial community structure during the period between days 15 and 30 that coincided with the total consumption of the labile C in the water-soluble OC pool. The FAME profiles during this period were characterised by high levels of hydroxy-fatty acids, also indicative of Gram-negative bacteria (Zelles 1999). It is interesting to note that there was a concomitant significant increase in the ^{15}N composition and a significant decrease in the ^{13}C composition of the SMB, suggesting that both of these are somewhat dependant on community structure. During the last five months of the incubation microbial communities had a higher proportion of Gram-positive bacteria, in particular actinobacteria, and fungi that are thought to be better decomposers of less labile compounds of soil organic matter (Elfstrand et al. 2008).

The ^{13}C analysis of FAME shows that the microbial isotopic enrichment during SOM decomposition also changed with the microbial community structure. During the phase of consumption of ^{13}C-enriched labile C of the water-soluble OC pool (up to day 15), FAME characteristic of Gram-negative bacteria were the most ^{13}C-enriched. After day 15, Gram-positive and fungal lipids became more ^{13}C-enriched (Figs. 5, 6). However, contrary to the total FAME profiles, there was a clear separation between the FAME $\delta^{13}C$ profiles measured on days 15 and 30, with lipids characteristic of Gram-positive bacteria being more ^{13}C-enriched on day 30. If changes in the $\delta^{13}C$ values of the FAME reflect changes in the active consumers, then it would mean that there is a certain lag between changes in the active consumer community structure and changes in the overall community structure. The interpretation of the isotopic composition of lipid biomarkers in long-term incubation experiments is often difficult due to the recycling of ^{13}C labelled compounds beyond the life-time of the first microbial generations (Amelung et al. 2008). Thus, the interpretation of the variations in the ^{13}C natural abundance of FAME may be somewhat speculative as neither the specific turnover rates of microorganisms nor the isotopic fractionation of the different metabolic pathways are known, both of which are essential for understanding the relationship between micro-organisms and substrate. Nevertheless, the relationships found here between the $\Delta^{13}C_{SMB/SOC}$ and the first PCA components FAME profiles or of FAME isotopic composition both suggest that the magnitude of the microbial isotopic fractionation is also influenced by the dynamics of the microbial communities.

Relevance of incubation studies

The objective of this work was initially to quantify the isotopic fractionation during a constant rate of SOM decomposition. Although there was a pre-incubation period of 2 weeks prior to the beginning of the monitored incubation, we observed far more variation than expected. For most of the variables, two different periods were distinguished: the first spanned the first 15–30 days of the incubation, during which most of the variability was observed. During the second period, which lasted from days 15–30 of the incubation until the end, the variables measured were much more constant, attaining an apparent steady-state. The variations observed at the beginning of the incubation are likely due to the perturbations associated with the setting up of the experiment (sieving prior to preincubation, wetting) as the soil was subjected to no other treatment (Franzluebbers 1999). These results indicate that the perturbations associated with experimental set-up can have an important effect on C and N dynamics, microbial ^{13}C and ^{15}N-fractionation and microbial community structure for up to 30 days. Therefore, one should be cautious when interpreting short-term studies, especially if no pre-incubation is performed. An approach to overcome the effect of the perturbation might be to use undisturbed, structured samples.

Another methodological consideration is related to the changes in $\Delta_{SMB/Min}$ observed during the incubation. This natural variation may have consequences for studies using ^{13}C isotope analysis to determine the origin of the $C–CO_2$ using either C3/C4 natural abundance (e.g. Nyberg et al. 2000) or the addition of labelled substrate (e.g. Fontaine et al. 2007). In order to determine the origin of the $C–CO_2$ in these types of studies, it is necessary to use the isotopic mass balance equation in which the $\delta^{13}C$ value of the native soil organic matter is generally determined in unamended control samples. There is a de facto underlying assumption that the $\delta^{13}C$ value will be the same in amended and unamended samples. However, the results obtained here suggest that this assumption may be violated. The addition of fresh organic matter is likely to alter the structure of respiring communities (Griffiths et al. 1999) and may also induce a shift in the type of substrate consumed (Fontaine et al. 2007), possibly leading to differences in microbial isotopic fractionation in amended and unamended samples. This is likely to be particularly problematic when the difference in ^{13}C abundance of the two different organic matters (i.e. added substrate and soil organic matter) is small.

Comparison with field studies

A consequence of the microbial isotopic fractionation during SOM decomposition is the progressive enrichment of the SOM in ^{13}C and ^{15}N as already mentioned in recent studies (Högberg and Johannisson 1993; Kramer et al. 2003; Dijkstra et al. 2006; Pörtl et al. 2007). This phenomenon was observed in bare-fallow

experimental plots adjacent to the plots sampled in this study. A ^{15}N enrichment of more than 5‰ was found nearly 70 years after the initiation of the long-term experiment along with a reduction of 55% of the N content (Bol et al. 2008). The stabilisation microbial derived ^{15}N-enriched molecules in combination with loss of ^{15}N depleted inorganic N by leaching or gas emission may continuously increase the SOM content in heavy isotopes. Based on the data of Bol et al. (2008), a simple mixing model with the assumption that the loss of N was linear give a ^{15}N depletion of the lost N compared to the SOM of approximately −4‰. This value is lower than that of the water soluble fraction measured in our study but it might correspond to that of the inorganic fraction (WSIN).

Over a 60 year period, the same bare-fallow soil showed a decrease in C content of approximately 60% and an increase of more than 1.6‰ of the soil $\delta^{13}C$ (Balesdent and Mariotti 1996). Two hypotheses have been proposed to explain this ^{13}C-enrichment with time. The first one is based on the isotopic fractionation associated with mineralisation. By fitting the relationship they found between C and $\delta^{13}C$ with Rayleigh's isotope fractionation equation, the authors estimated the fractionation coefficient associated with C mineralization ($\Delta_{Min/SOC}$) at −1.71‰, which is close to that found in our study during the steady state period ($\Delta_{Min/SOC}$ ranging from −1.1‰ to −1.5‰, for the final 5 months of the incubation). A ^{13}C discrimination factor of 1 to 2‰ was also estimated using a Rayleigh distillation model to fit the relationship between the C content and $\delta^{13}C$ value on depth profile of soils (Wynn et al. (2005, 2006). The second hypothesis is that ^{13}C depleted components of the SOM are preferentially mineralised. Our results do not support the idea that soil microorganisms preferentially use isotopically depleted substrates as at the beginning of the incubation ^{13}C enriched substrate tended to be used.

Conclusion

Once the microbial communities reached a steady-state, the isotopic trends appeared to corroborate those obtained in long term experiments in the field in that there is a constant microbial isotopic fractionation leading to an isotopic enrichment of the SOM over the long-term. This type of experimental data, obtained from medium-term incubations, could be used to inform models constructed to explore the relationships between various soil C pools (e.g. Gregorich et al. 2000). The work presented here shows, for the first time, that ^{13}C and ^{15}N fractionation are linked not only to C and N dynamics but also to the dynamics of microbial community structure, i.e. the temporal succession of the different microbial decomposers. These variations are indicators of different processes occurring in soil, such as changes in N metabolism or different substrate-C usage, and show that both are linked. A rather surprising result obtained was that the setting up of the experiment appeared to have a prolonged effect on microbial, C and N dynamics and should be taken into account, in particular in short-term studies.

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Appendix

The use of FAME rather than PLFA may alter the resolution of the microbial community snapshot. FAME include lipids from sources other than live microbial biomass such as dead microbial biomass (Drenovsky et al. 2004) and their half-lives are greater than both PLFA and total biomass (Kindler et al. 2006, 2009). Here, the total amount of FAME changed significantly with time ($P < 0.001$), reaching a maximum of 177 ± 24 nmoles g^{-1} soil on the 8th day of incubation and a minimum of 136 ± 10 nmoles g^{-1} soil after 6 months. Figure 8a. shows the relationship between the total amount of FAME and the amount of microbial biomass estimated by fumigation-extraction (expressed in C equivalent: C_{SMB} and $C_{\sum FAME}$). A good linear correlation was found with $C_{\sum FAME} = 0.15 \times C_{SMBs} + 4.78$ ($n = 30$, $r^2 = 0.85$, $P < 0.001$). The intercept was not significantly different from zero. A good linear correlation was also found between the ^{13}C composition of the microbial biomass and the overall ^{13}C composition of FAME (Fig. 8b): $\delta^{13}C_{\sum FAME} = 0.95 \times \delta^{13}C_{SMB} - 3.11$ ($n = 30$, $r^2 = 0.67$, $P < 0.001$). The intercept was not significantly different from zero. This was corroborated by the lack of

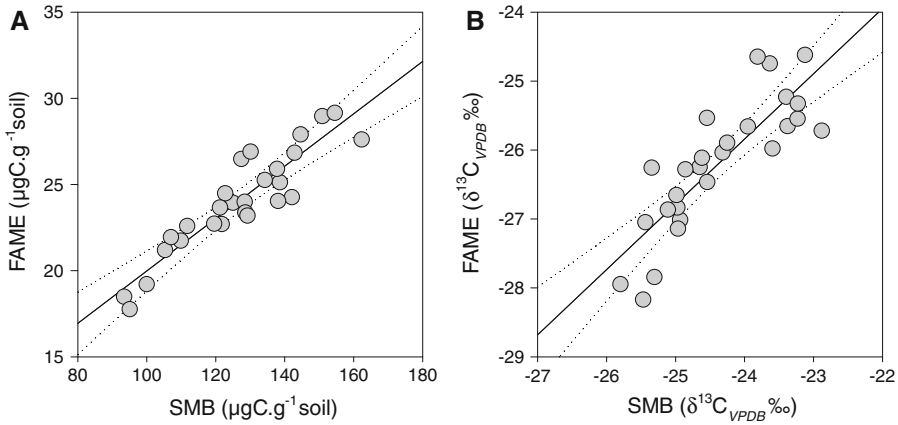

Fig. 8 Relationship between the amount of FAME and the Soil Microbial Biomass expressed in µgC g^{-1} soil (**a**) and between the overall $\delta^{13}C$ value of FAME ($\delta^{13}C_{\sum FAME}$) and the $\delta^{13}C$ value of soil microbial biomass ($\delta^{13}C_{SMB}$) (**b**). The dark line represents the linear regression and the dotted lines represent the 99% confidence intervals

significant difference in the $\Delta^{13}C_{\sum FAME/SMB}$ throughout the incubation (Table 1). The strong correlations between the total amount of FAME and the microbial biomass measured by fumigation extraction and between the ^{13}C composition of the SMB and the overall composition of the FAME demonstrated the pertinence of the FAME approach for studying changes in soil microbial community structure.

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